NOTES ON THE HISTOLOGY OF THE ALMOND[1]

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INTRODUCTION

In connection with a study of one of the technological problems involved in the salting of domestic almonds, some rather interesting data on the histology of the almond kernel or seed have been assembled. This material is presented here not only on account of its general interest to histologists, but because it may afford some assistance to the trade in developing a method for the determinination of types if not of varieties of shelled almonds as they appear in the open market.

MATERIAL

The material studied[2] consists of four varieties of domestic almonds and four varieties of imported almonds. The domestic almonds, Nonpareil, Ne Plus Ultra, I. X. L., and Drake, are among the leading varieties grown in California, and the imported nuts, Etna, Marcona, Valencia, and Alicante, originally brought from Italy and Spain, are largely used by bakers and confectioners. The domestic nuts are paper and soft shelled, while the imported nuts are standard and hard shelled. On the basis of the hardness of the shell, these samples would be classed as follows:[3]

Paper	I. X. L. Ne Plus Ultra. Nonpareil.
Soft	Drake.
Standard	Marcona.
Hard	Valencia. Etna. Alicante (?).

The almond fruit is a typical drupe; that is, it consists of a single seed surrounded by a stony endocarp which forms the shell, a fleshy mesocarp, and an outer skin or epicarp. The mesocarp is tough and leathery and, with its protecting epicarp, splits away from the endocarp at maturity. The difference between hard and soft shelled almonds lies in the difference in texture of the endocarp. The shelled almond, or kernel, consists of the embryo, surrounded by the modified tissues of the ovule. The embryo itself is made up of a small, pointed radicle, a delicate plumule, and two straight massive cotyledons whose cells are closely packed with reserve food material in the form of oil droplets and protein granules. The surrounding tissues consist of the remnants of the endosperm and nucellus, and the integuments.

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[2] This material was furnished by the California Almond Growers' Exchange to whom the writer is greatly indebted.

[3] Based on General Information on Almonds, Foodstuffs Division, Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce, 1928. [Mimeographed.]

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REVIEW OF LITERATURE

Early work on the structure of the almond was done with a view to distinguishing microscopically between the kernels of almonds and such related seeds as peaches, apricots, and plums, which were being substituted for almonds, both in almond paste and in the expressing of the oil. Early workers on the microscopy of the almond seed agreed that there were no differences in structure between bitter and sweet almonds (*1*).[4] The question of a difference between hard and soft shelled almonds seems not to have been raised until Young (*13*) contrasted them. His opening statement, however, is, "The bitter almond and the hard and soft or paper shelled varieties of the sweet almond all belong to one species and are hence very similar in structure." Young's work is the most detailed histological study of the almond published in English. Meyer (*6*) gives a careful description of the development of the seed from fertilization to maturity, with a discussion of the various tissues in the ripened seed and seed coat. Tschirch (*10*) bases his discussion on the best of the earlier workers and adds several new points, especially along the line of microchemical tests for cell-wall substances. Kraemer (*5*) offers probably the best summary in English of later foreign work, with well-executed line drawings, which are for the most part adaptations from other writers. Winton (*11*) seems to be the only other American writer who has published work on this subject.

METHOD

Preliminary examination having shown that in all probability the greatest anatomical differences between varieties or types of nuts were to be found in the "skin" or tissues surrounding the embryo, the structure of these tissues was worked out in some detail. Skins removed by blanching, or soaking for two or three minutes in boiling water, were boiled in 2 per cent potassium hydroxide and allowed to stand overnight, or until shrunken tissues and "obliterated cells" were sufficiently swelled so that their shape, size, arrangement, and relationships could be determined. Following this treatment, the aleurone layer could be stripped from the inner surface of the testa, the epidermal stone cells or hairs could be scraped from the outer surface with a dull scalpel, and the remaining tissues could be teased apart with needles or fine-pointed tweezers. Material was also embedded in paraffin, sectioned 10 microns thick, and stained with safranine and light green, using Land's schedule as given by Chamberlain (*2*). In addition to differentiating cellulose and lignin in the testa, this stain proved particularly satisfactory for the storage tissue of the cotyledons, the cell walls staining green and the protein granules bright red. Microchemical tests used were: For lignin, phloroglucinol-hydrochloric acid; for cellulose, chlorzinciodide; for cutin and oil, Sudan III; for tannin, ferric chloride; for protein, iodine in potassium iodide and the xanthoproteic reaction.

THE SEED COAT

The inner white layer stripped away from the brown or yellowish-brown testa after maceration in 2 per cent potassium hydroxide con-

[4] References made by number (italic) to Literature Cited, p. 800.

sists almost entirely of aleurone and obliterated cells; that is, endosperm and nucellus or perisperm. The aleurone cells of the endosperm (fig. 1, A, 2, *al*) are thick walled, quadrilateral to hexagonal

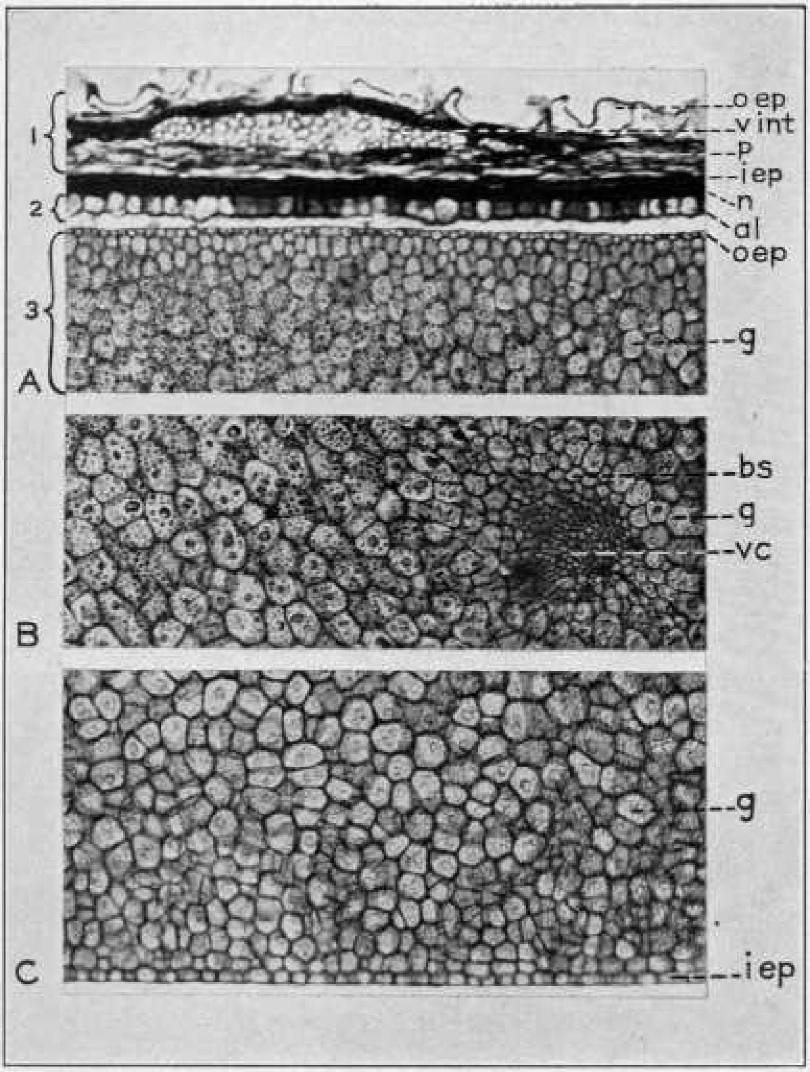

FIGURE 1.—Cross sections of Nonpareil almond. × 145. A, Testa and outer portion of cotyledon: 1, Integuments: *o ep*, Outer epidermis; *p*, flattened cells of parenchyma; *v int*, vascular bundle; *i ep*, inner epidermis; 2, nucellus and endosperm; *n*, obliterated tissue of nucellus; *al*, aleurone layer; 3, cotyledon: *o ep*, outer, or lower, epidermis; *g*, ground tissue with proteing ranules. B, Central portion of cotyledon: *g*, Ground tissue; *b s*, bundle sheath; *v c*, vascular bundle. C, Inner portion of cotyledon: *g*, Ground tissue; *i ep*, inner, or upper, epidermis

in surface outline, usually somewhat elongated in the direction of the long axis of the seed, and without intercellular spaces. Each cell contains one or more large oil globules and numerous fine protein granules. The radicle is embedded in a mass of aleurone cells, but over the flat surface of the cotyledons the layer is usually one cell

thick, although it may be two or even three cells thick. The thickened areas may be due to tangential division of the cells, but in some instances they seem to be due to infoldings, the folds being parallel to the long axis of the seed. Individual aleurone cells vary in size and shape in different regions and in different varieties of nuts, but these differences are not significant in the determination of varieties. A thin layer of obliterated cells can sometimes be found between the two cotyledons or on the inner surface of the aleurone layer. This is composed of fragmentary traces of thin-walled endosperm tissue. It is especially evident in the chalazal region. Its presence between the cotyledons was first noted and figured by Hartwich (*4*).

The perisperm or nucellus (fig. 1, A, 2, *n*) consists of obliterated tissue, in which the individual cells are faintly visible after prolonged treatment with potassium hydroxide. They are very large, roughly 150 by 400 microns, with extremely delicate colorless walls. The outermost layer or epidermis of the nucellus consists of narrow, elongated cells with faintly beaded walls, regularly arranged with their long diameters parallel with the long axis of the seed. They are approximately 50 by 150 microns, although varying widely from these figures, which are the average of 125 measurements on four varieties, two domestic and two foreign. The variation in a single piece of tissue is often as great as in the different types of nuts.

The testa, or outer portion of the seed coat, is of integumentary origin. It is this part of the almond and related seeds that has received most attention from students of histology. Several points in structural anatomy have not yet been cleared up, and this still remains a fertile field for study.

The inner epidermis (fig. 1, A, 1, *i ep*) consists of small flat cells, roughly quadrilaterial in surface view, with finely sinuate walls. These cells appear to be cutinized on their outer surface, the layer of cutin separating them from the extremely thin-walled epidermis of the nucellus. This interpretation is contrary to the opinion of Moeller and Thoms (*8*), who state that the cuticularized membrane belongs to the inner tissues which lie in contact with the brown integumentary layer, but is in agreement with that of Péchoutre (*9*), who states that the inner epidermis of the seed coat is slightly thickened and cutinized. The cell content forms a solid platelike mass, highly refractive, dark brown in color, and soluble in hot water and in 2 per cent potassium hydroxide, but insoluble in alcohol and ether.

The body of the testa is made up of flattened parenchyma cells (fig. 1, A, 1, *p*) separated into an inner and outer region. The inner region is made up of cells with colorless cellulose walls. The outer region consists of cells with lignified yellowish-brown walls. Between the two regions are the vascular bundles or veins (fig. 1, A, 1, *v int*) radiating from the chalaza and running practically parallel throughout the length of the seed to the micropylar tip. Along the veins are found rosette crystal aggregates of calcium oxalate, more abundant in hard-shelled types. The parenchyma cells of both the inner and outer regions when swelled in 2 per cent potassium hydroxide form a loose spongy tissue, the individual cells usually being in direct contact only by means of short blunt processes. Hence there is a large amount of intercellular space which is not apparent in the normal flattened condition of the cells in the ripened seed coat. The cells of the exterior subepidermal layers are much smaller, but are also

loosely arranged with much intercellular space. (Fig. 3, A, *c*.) The development of the integuments during the maturing of the seed has not been followed, and no statement appears in the literature consulted as to where the boundary between the two integuments lies. Péchoutre's sketches indicate that the tissues of the inner integument are more collapsed than those of the outer. It is natural to assume that the inner cells with cellulose walls are morphologically a part of the inner integument and the outer lignified cells are a part of the outer integument.

The outer epidermal layer of the testa, which forms the surface of the seed, has been subjected to profound modification. Most of the cells are enormously enlarged, and in many the walls are thickened and lignified. (Figs. 2, 3, and 4.[5]) The phloroglucinol-hydrochloric acid reaction shows the distribution and relative abundance of the lignified cells very clearly. (Fig. 4, B.) These lignified epidermal cells have attracted the attention of histologists for many years. They have been variously described as hair cells, barrel cells (Tonnenzellen), or balloon cells (Kugelzellen), but are usually spoken of as stone cells. They are described by the early writers as egg-shaped or globular, though Young (*13*) figures extremely elongated cells, and Winton (*11*) states that they are commonly higher than broad and often elongated, like trichomes. In fact, there has been some discussion as to whether they are to be considered as epidermis or as trichomes.

The cell lumen is empty, but a thin protoplasmic layer is closely appressed to the inner surface of the cell wall. This is apparently what Tschirch (*10*) considers an inner layer of cell-wall substance, which he differentiates from the lignified portion of the wall. This layer gives a deep-blue reaction with ferric chloride, indicating the presence of tannin.

The epidermal cells vary noticeably in size, shape, degree of lignification, and distribution of lignified cells (figs. 2, 3, and 4), not only as between hard and soft shelled almonds, which has been noted by several writers, but also as between the varieties in either class, a fact which seems to have escaped the notice of earlier observers. In fact, Berg (*1*) states that there are no differences in structure between sweet and bitter almonds, the only difference between the two being in the cell contents of the kernels. Young (*13*) describes and figures the stone cells of the epidermis of the integuments of hard-shelled almonds, but says that in soft-shelled almonds a few of the epidermal cells have thicker walls than others, suggesting the stone cells found in the epidermis of the hard-shelled nut. Winton (*11*) agrees with Young. No varietal comparisons seem to have been made.

Wittmack and Buchwald (*12*) seem to have observed only the lignified cells and describe them as forming a discontinuous layer in the ripe seed. Hannig (*3*) first described the thin-walled nonlignified cells which are interspersed with the lignified cells, and remarked that they are sometimes found unaltered, but frequently are collapsed, and usually are completely crushed or altogether destroyed. This, he observed, gives the lignified cells the appearance of standing out from the surface singly or in groups; hence the opinion of earlier writers that they are hairs.

[5] The writer is greatly indebted to G. L. Keenan, microanalyst, Food and Drug Administration, U. S. Department of Agriculture, for assistance in the preparation of these figures.

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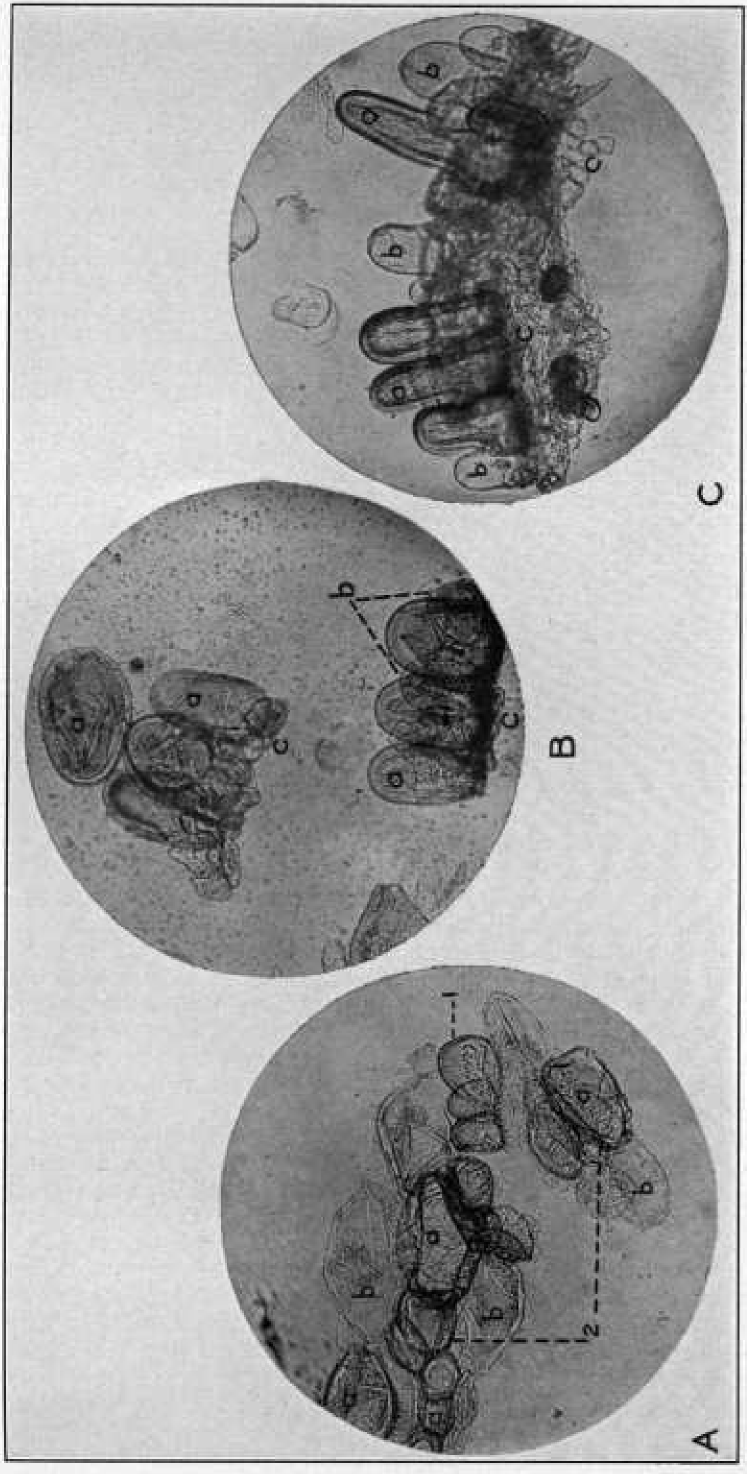

FIGURE 2.—A, Outer epidermis of testa of Nonpareil almond: 1, Group of three stone cells from the side, showing heavy walls and pores; 2, surface view: *a*, Stone cells; *b*, thin-walled cells. B, Epidermal cells of Drake almond: *a*, Stone cells; *b*, thin-walled cells; *c*, subepidermal cells. C, Group of cells from Marcona almond: *a*, Stone cells; *b*, thin-walled cells; *c*, subepidermal cells. Macerated material, unstained. × 80

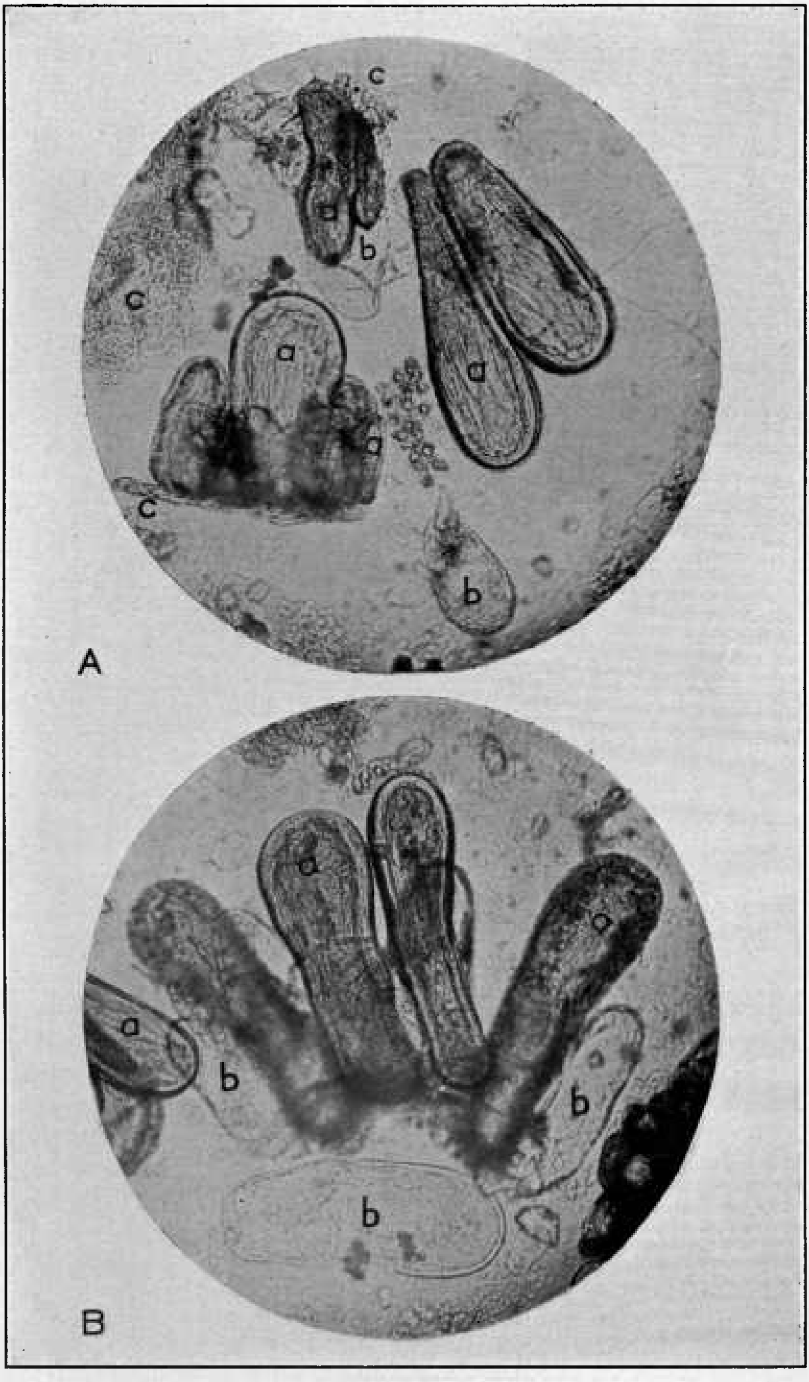

FIGURE 3.—Etna almond. A, Various types of cells from the testa: *a*, Stone cells of outer epidermis; *b*, thin-walled cells of outer epidermis; *c*, subepidermal cells. B, A group of very large epidermal cells: *a*, Stone cells; *b*, thin-walled cells. Macerated material, unstained. × 80

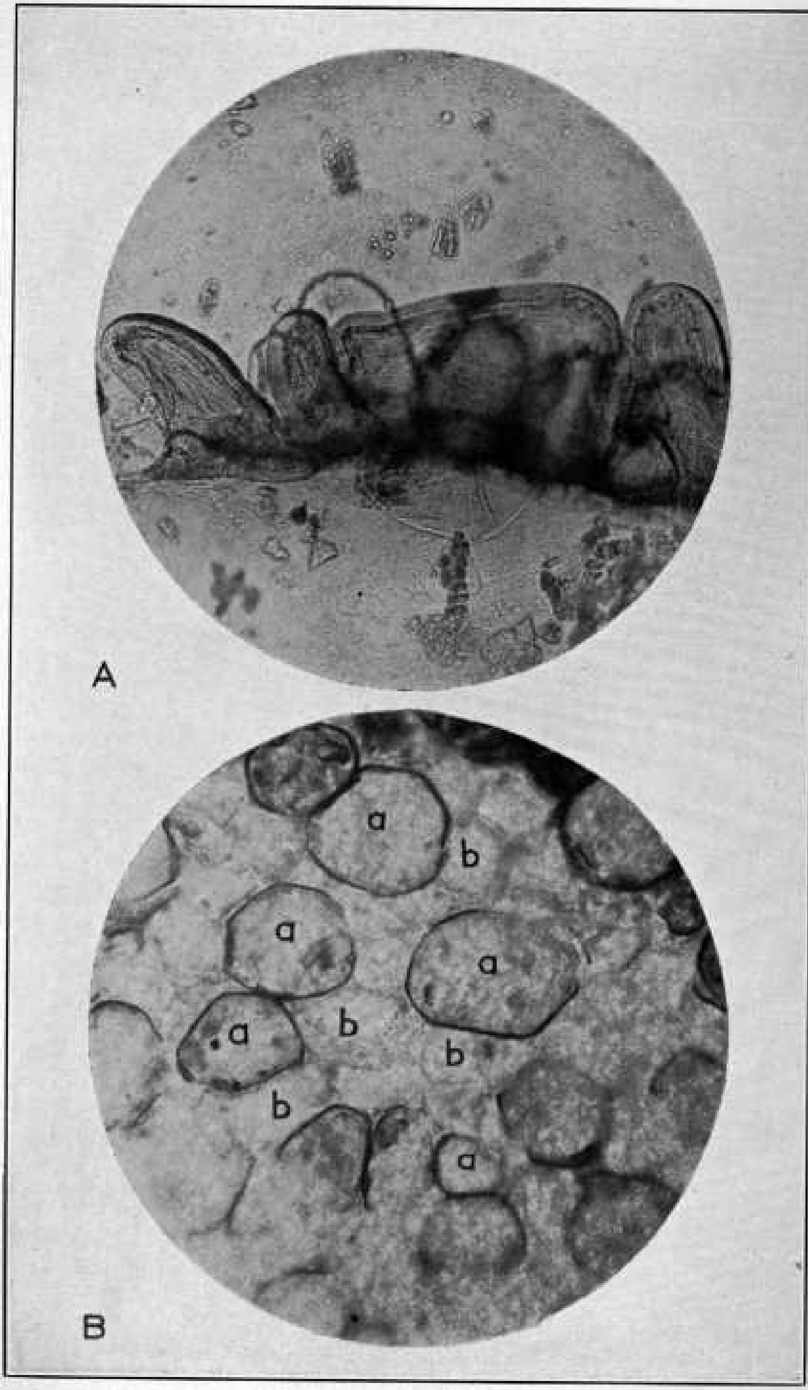

FIGURE 4.—Etna almond. A, Epidermal stone cells, over a vein, showing modified shapes. Macerated tissue, unstained. × 80. B, Surface view of testa: *a*, Stone cell; *b*, thin-walled cell. Stained with phloroglucinol-hydrochloric acid. × 80

The present study shows that in the varieties examined thick-walled lignified cells occur in the epidermal layer of the testa in both soft and hard shelled almonds. The lignified cells do not differ from the unlignified in size or shape, and are distributed irregularly over the surface, being apparently more closely crowded and more elongated over the radicle, and especially along the base of the raphe. Over the veins, and especially along the raphe, they often assume bizarre shapes as if stretched by the increasing growth of the seed. Over the chalaza the cells are much smaller.

The bases of the cells where in contact with each other and with the subepidermal layer are porous, the pores apparently connecting the cavities of adjacent cells. These pores are simple and unbranched, and usually round, but they may be elliptical, and several may be ranged at irregular intervals along shallow crescentic or serpentine grooves in the cell wall.

The free surface of the cell may be slightly domed or enormously extended, smooth on the outer surface and punctate or channeled on the inner. The elongated cells may be balloonlike, nipplelike, or beaked, and many of the individual cells are so large as to be plainly visible through a hand lens. Their rounded extremities give the roughened granular appearance to the shelled nut.

In the varieties of soft-shelled almonds studied the walls of the lignified cells are faintly colored a yellowish brown, and are thickened very little; the pores are comparatively few and aggregated in groups, and the inner surface of the outer wall is not punctate or ridged. (Fig. 2, A and B.) In all four varieties they give a very definite phloroglucinol-hydrochloric acid reaction. In the Nonpareil (fig. 2, A, 1), which appears smooth to the naked eye, the outer wall is only slightly domed, the depth of the cell being approximately equal to its diameter and averaging about 85 microns. In I. X. L. and Drake (fig. 2, B) the cells average approximately 130 microns in height and 100 microns in diameter, although cells were found more than 450 microns in height or diameter, such cells being easily visible with a hand lens. In Ne Plus Ultra the cells average approximately 175 microns in height and 135 microns in diameter, some cells measuring more than 450 microns.

In the hard-shelled almonds the lignified cells have heavily thickened walls, especially the free outer walls, which show stratification in all varieties when swelled in 2 per cent potassium hydroxide. They are strongly colored brown or yellowish brown. The Marcona (fig. 2, C) has the smallest cells measured, averaging approximately 130 microns in height and 75 microns in diameter, although reaching to from 300 to 370 microns in some instances. In the Etna (figs. 3 and 4) occurs the most marked elongation, the cells averaging approximately 300 microns in height and 130 microns in diameter, some cells being 650 microns in height, some 500 microns in width. In Valencia the cells average approximately 240 microns in height and 130 microns in diameter, with some cells exceeding 500 microns in either height or diameter. In Alicante the average measurements approximate 150 microns for height and 100 microns for diameter, although an occasional cell reaches 430 microns in height or 650 microns in diameter, the greatest diameter measured in any of the varieties.

In other words, the ratio of height to width in the four varieties of soft-shelled almonds averages 5: 4, whereas in the four varieties of hard-shelled almonds the average ratio is 2: 1. It must be borne in mind that extremes in height and diameter are not necessarily found in the same cell. Extremely elongated cells (fig. 3, B) may be found over any portion of the surface, with the exception of the chalaza, and they are especially abundant toward the apical end and along the margins of the raphe. Cells with the diameters stretched parallel to the long axis of the seed (fig. 4, A) are found immediately over the veins, and especially over the raphe. The averages given are based on at least 200 measurements for each variety.

A comparison of figures obtained in this study with those given by earlier workers is of interest. Such a comparison is presented in Tables 1 and 2.

TABLE 1.—*Measurements of lignified cells of integuments of hard and soft shelled almonds*

DOMESTIC ALMONDS, SOFT SHELLED

Variety	Height (μ)			Diameter (μ)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Nonpareil	35	163	83	35	185	87
I. X. L.	43	483	131	21	490	97
Drake	28	454	131	21	440	107
Ne Plus Ultra	21	483	177	28	461	136
Average			130			106

IMPORTED ALMONDS, HARD SHELLED

Variety	Height (μ)			Diameter (μ)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Marcona	35	319	132	14	369	75
Etna	57	653	310	21	497	133
Valencia	35	547	241	21	516	132
Alicante	43	433	153	21	653	98
Average			209			109

TABLE 2.—*Measurements of lignified cells of integuments of almonds*

[Taken from the literature]

Author	Year	Variety	Height (μ)			Diameter (μ)		
			Minimum	Maximum	Average	Minimum	Maximum	Average
Wittmack & Buchwald (*12*)	1901		90 136	109 159		69	84	100
J. Moeller (*7*)	1905	Italian			175			
E. Hannig (*3*)	1911		64	144 (200)		23		
A. Tschirch (*10*)	1912		120	335		70	135	
W. J. Young (*13*)	1912	Soft				100	200	
		Hard		400				
A. L. Winton (*11*)	1916	Hard	60	400	100+			
H. Kraemer (*5*)	1928		70	175		65	100	

THE EMBRYO

In the dormant embryo of the ripened seed the massive cotyledons are usually flat and closely appressed, but an occasional seed is found in which one cotyledon is folded on itself longitudinally and the other is wrapped about it more or less completely. In most such cases the inner cotyledon is smaller, and it may be much underdeveloped or possibly even atrophied as a result of pressure from the surrounding cotyledon, which has a distinct advantage so far as expansion due to growth is concerned.

The cells of the ground tissue are fairly large and are packed with stored food material, each cell containing from one to several large protein granules and many small ones (fig. 1, A, B, and C, *g*), in addition to oil and soluble carbohydrates. In some cases there appear to be areas of physiological breakdown in otherwise normal cotyledons, the cells appearing empty and collapsed under the microscope.

The system of venation is well developed, forming a network which is very evident in longitudinal sections. The epidermal cells of the inner and outer surfaces of the cotyledon, corresponding to the upper and lower surfaces of a leaf, respectively, are small and narrow, the long axis of the cell being parallel with the long axis of the cotyledon. The outer or lower epidermal cells are much smaller than the inner and upper ones. Young's photomicrograph (*2*) is apparently a surface view of the outer epidermis. The difference in size of the two epidermal layers is well shown in Figure 1, A, 3, *o ep*, and C, *i ep*. This figure also shows the contrast in size between the cells of the epidermal layers and those of the ground tissue. The cell walls in all embryo cells are unmodified parenchyma and are extremely thin. Modifications extend only to size, shape, and content, with the exception of the beginning of thickening in the tracheal tissue of the veins.

SUMMARY AND CONCLUSIONS

A comparative study of the histology of four varieties of domestic soft-shelled almonds and four imported hard-shelled varieties is reported.

Evidence is presented to show that the chief difference between the two types of nuts lies in the structure of the cells of the outer epidermis of the testa, or the surface of the seed coat.

The modified epidermal cells of soft-shelled almonds are lignified, but only slightly as compared with those of hard-shelled almonds. They can therefore be considered as poorly developed stone cells.

Careful measurements indicate that in addition to being heavier walled the stone cells of the seed coat of hard-shelled almonds are more elongated than those of soft-shelled almonds, the ratio of length to width in hard-shelled almonds being approximately 2 to 1 and in soft-shelled almonds 5 to 4.

The importance of this study of the variation in the surface cells of the testa lies in the fact that with further examination and correlation of data it may be possible to develop an easily workable method for distinguishing the different varieties of almonds in the shelled condition, thus detecting or preventing misrepresentation or adulteration.

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